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Short Communication

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Laboratory evaluations of biodegradable boric acid hydrogel baits for the control of Argentine ant (Hymenoptera: Formicidae)

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Due to their mutualistic relationship with plant pests, the Argentine ant is considered a major pest in subtropical fruit orchards and vineyards. Besides insecticide sprays, liquid baiting has been demonstrated as an effective method to suppress the Argentine ant populations. To improve the economic feasibility of liquid baiting, hydrogel materials have been recently tested as a carrier for liquid baits containing various insecticidal active ingredients. Here, we tested boric acid as a toxicant in the aqueous sugar bait delivered in a biodegradable calcium alginate hydrogel. Laboratory tests demonstrated that boric acid (1%) liquid bait incorporated in the calcium alginate hydrogel effectively killed Argentine ant workers. Potassium sorbate (0.25%) added to the liquid bait as a preservative did not impact the efficacy of boric acid even though it significantly reduced the degree of swelling of the hydrogel beads in the bait solution. Testing with 2-month-old bait suggested that long-term storage might impact bait efficacy even with potassium sorbate preservative.

Key words: boric acid, calcium alginate, hydrogel, ant bait, *Linepithema humile*

Introduction

In subtropical fruit orchards and vineyards, Argentine ants *Linepithema humile* (Mayr) tend honeydew-producing hemipteran pests and prevent their natural predators from attacking them (Tillberg et al. 2007), hindering biological control (Buckley 1987). Hence, managing Argentine ants is an important part of the integrated pest management (IPM) of honeydew-producing pests. Argentine ant control in agriculture often requires chemical options (Klotz et al. 2003, Silverman and Brightwell 2008). Due to concerns associated with spray insecticides (e.g., environmental contamination and nontarget impact) (John and Shaike 2015), liquid baiting has been explored as an alternative strategy to control Argentine ants (Rust et al. 2000, Gore and Schal 2004, Daane et al. 2006, Cooper et al. 2008).

However, conventional liquid baiting necessitates the use of bait stations, a financial hindrance on liquid baiting at larger scales (Daane et al. 2008). Accordingly, hydrogel compounds were tested as a carrier of liquid bait. Different hydrogel compounds have been tested, initially synthetic polyacrylamide (Boser et al. 2014, Buczkowski et al. 2014, Rust et al. 2015), and more recently, biodegradable calcium alginate (Tay et al. 2017). However, most of these efforts used thiamethoxam as a bait toxicant (Boser et al. 2014, Buczkowski et al. 2014, Rust et al. 2015, Tay et al. 2017, McCalla et al. 2020).

Boric acid has been extensively tested as an active ingredient for ant baits (Klotz et al. 1998, Rust et al. 2004, Greenberg et al. 2006). Useful features of boric acid for baiting include being soluble at low concentrations in water and having delayed activity when ingested by ants (Klotz et al. 1997). Also, boric acid used at insecticidal levels is considered nontoxic to noninsect invertebrates and vertebrates (US EPA 1993). When used in bait stations, boric acid can be used in organic agricultural settings (Greenberg et al. 2006). Cooper et al. (2019) tested 0.5% boric acid liquid baits in synthetic polyacrylamide hydrogel to suppress Argentine ants in California vineyards. Boric acid bait in calcium alginate hydrogel has been tested in urban residential settings (Choe et al. 2021), but not in agricultural settings.

This laboratory study examined the following to determine the composition of the calcium alginate hydrogel bait with boric acid for a field test. First, boric acid samples from two different suppliers, U.S. Borax Inc. (Boron, CA) and Sigma-Aldrich (St. Louis, MO), were compared. Since Sigma-Aldrich was the source of boric acid in a previous hydrogel trial (Choe et al. 2021), the comparative study was necessary to ensure the boric acid from a new source was comparable. Secondly, the current study tested if potassium sorbate, a preservative, had any impact on the efficacy of the boric acid hydrogel bait. The addition of a preservative in the bait was necessary for real-world applications, where the manufactured bait may need to be stored for some time before being applied in the field (Qin et al. 2017). Current study also quantified how the swelling of the hydrogel was affected by these two different solutes (i.e., boric acid and potassium sorbate) (Golmohamadi and Wilkinson 2013). Finally, the current study tested if long-term storage of the bait impacted bait efficacy.

Materials and Methods

Experimental Colony

Argentine ants were collected from the field and kept in laboratory conditions following methods described in Welzel and Choe (2016). Clear plastic cups were used as colony containers for experiment. Details of experimental colony design are provided in Fig. 1.

Ants from the stock colony were placed into a plastic box containing several 5-cm sections of vinyl tubing (8 mm ID, 11mm OD). Once the ants (queens, brood, and workers) aggregated in these 'transfer tubing' sections, each of the transfer tubing sections was emptied into a colony container. This process prepared many colony fractions of comparable sizes and compositions quickly. For example, the average worker count per experimental colony was 479 ± 10 (mean ± SEM, n = 90). Visual inspection ensured that each experimental colony had at least one queen. After setting up the experimental colonies, 50 µL of 25% (wt/vol) sucrose water was provided per colony. The colonies were tested with hydrogel baits on the third day.

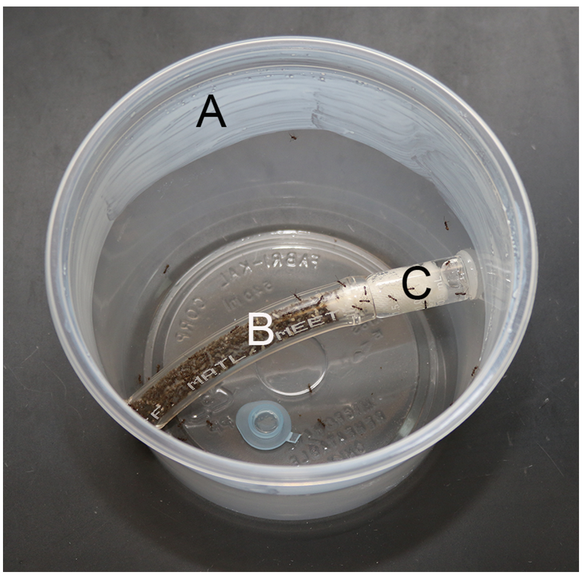

Fig. 1. Experimental colony. Clear 473-mL plastic cups (PK16S-C; Frabri-Kal, Kalamazoo, MI) were used as A) colony containers, B) A 7-cm section of clear vinyl tubing (8 mm ID, 11 mm OD, Tygon, Saint-Gobains Performance Plastics, Akron, OH), C) fitted with a 2-mL vial (Agilent, Santa Clara, CA) containing deionized water and a 2.5-cm cotton wick plug (Absorbal, Wheat Ridge, CO) served as a nest tubing. The nest tubing was affixed to the interior of the colony container by attaching the bottom of the vial to the inner surface using hot glue.

Bait Preparation

For comparison, several different bait solutions with 25% (wt/vol) sucrose were prepared. Boric acid samples from two different sources, Technical Powder Optibor (>99.9%) (U.S. Borax Inc., Boron, CA) [BA(O)], and Boric Acid ReagentPlus (≥99.5%) (Sigma-Aldrich, St. Louis, MO) [BA(S)], were included in the experimental design. A representative preservative, potassium sorbate ReagentPlus (99%) (PS) (Sigma-Aldrich), was also included. Overall, the treatments tested were BA(O), BA(O) + PS, BA(S), and BA(S) + PS in sucrose solution. Sucrose only (C) or sucrose with potassium sorbate (C + PS) were included as controls. Concentrations for BA and PS were 1 and 0.25 % (wt/vol), respectively.

Calcium alginate hydrogel beads were produced via the methods described by Tay et al. (2017). The hydrogel beads were soaked in a bait solution to prepare the hydrogel bait (a process referred to as 'conditioning'). For each treatment, 100 ml of conditioning solution was used to soak 5 hydrogel beads (approximately 0.3 ml). Since the amount of conditioning solution was much larger than the amount of water in the initial hydrogel beads, any dilution factor was considered negligible. The hydrogel beads were kept in the bait solution for 3 days (at 4°C) until testing. All solutions were prepared with deionized water.

Swelling Analysis

Degree of swelling of hydrogel beads over the conditioning process was measured by weighing a same set of 5 hydrogel beads before conditioning (initial weight) and after conditioning (final weight). Since the initial weight values were highly uniform, final weights were compared between different treatments. This experiment was replicated 15 times.

Efficacy Test

Immediately before treatments, all dead ants were removed from the experimental colonies. The hydrogel baits (2–3 beads) were provided in a small cup (lid from 1.5-mL centrifuge tube, Sigma-Aldrich) placed at the bottom of the experimental colony. As the hydrogel baits lost moisture over time, they were rehydrated with ≈0.1 ml of deionized water every other day until the end of the experiment. Experiments were replicated either 11 (for treatments with PS) or 15 (treatments without PS) times.

Table 1. Swelling of five hydrogel beads when conditioned in different bait solutions with different combinations of boric acid (BA) and potassium sorbate (PS) preservative. Details on treatments are provided in the text. All values are mean ± SEM. For the final weight column, different letters represent groups with significant differences (ANOVA with Tukey's HSD; α = 0.05). Percent change was calculated by (final wt—initial wt)/initial wt × 100

Treatment	Initial wt (g, n = 90)	Final wt (g, n = 15)	Percent change (%)
BA(O)	0.31 ± 0.00	1.20 ± 0.01a	287
BA(O) + PS		0.86 ± 0.01b	177
BA(S)		1.00 ± 0.02c	223
BA(S) + PS		0.80 ± 0.01d	158
C		1.90 ± 0.02e	513
C + PS		0.88 ± 0.01b	184

Using final bait composition [BA(O) + PS, see results], the aged bait was tested to determine if there was any impact of long-term storage on bait efficacy. The aged bait was prepared by storing the freshly made bait in a 19.4 liter bucket with a lid at 24°C for 2 months. The mortality from the aged bait at day 14 was compared with that from the corresponding fresh bait. This experiment was replicated 12 times.

With the boric acid bait treatments, Argentine ant mortality occurs slowly (Rust et al. 2004), and many ants succumb within the nest tubing (B. Le, unpublished data). By day 14, the surviving ants piled the dead ants outside the nest tubing, allowing accurate mortality counting.

Statistical Analyses

For the swelling analysis, log-transformed final weights [ln(x + 1)] were first analyzed using ANOVA, and followed up with Tukey's HSD post hoc test at α = 0.05 (Analytical Software 2013). Kruskal–Wallis test was used to compare the mortality data between different

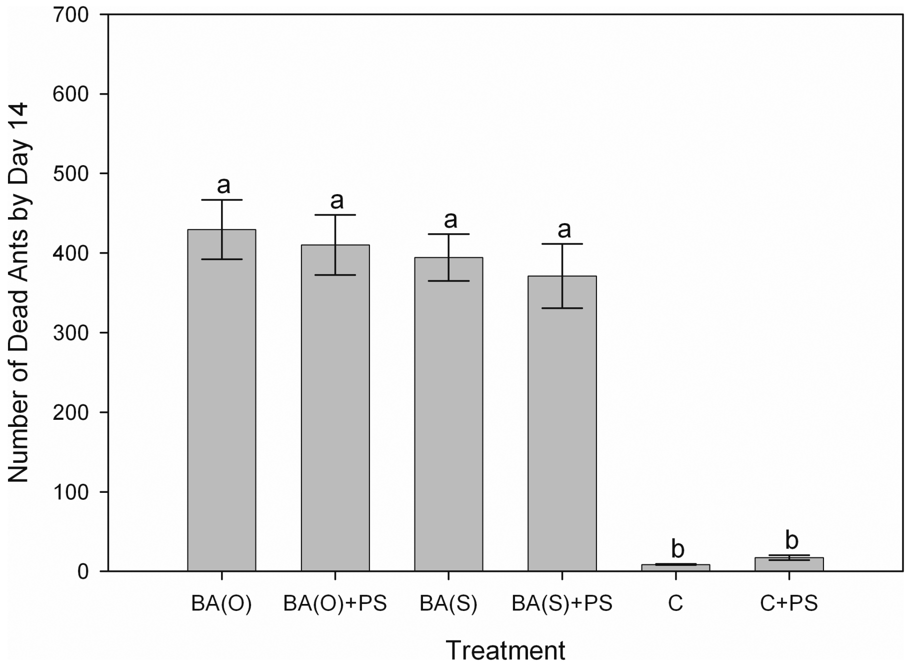

Fig. 2. Comparison of average number of dead ants per cup for each treatment. Error bar indicates standard error of means (SEM). Different letters above bars represent groups with significant differences (Kruskal-Wallis test with Dunn's all-pairwise comparisons test; α = 0.05).

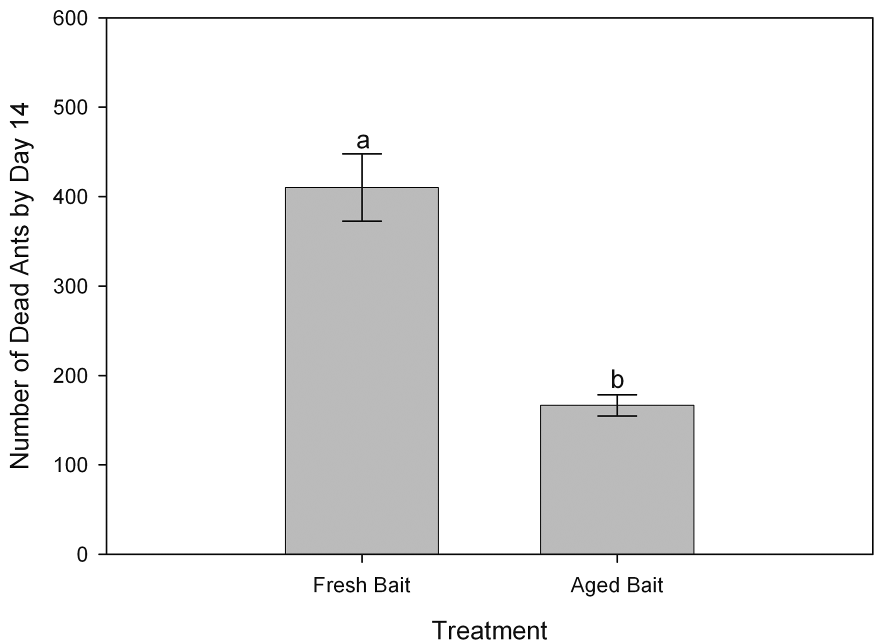

Fig. 3. Comparison of average number of dead ants per cup at day 14 between fresh and aged bait [BA(O) + PS]. Error bar represents standard error of means (SEM). Different letters above bars denote significant differences (Wilcoxon rank sum test; α = 0.05).

treatments. Once the null hypothesis was rejected, Dunn's all-pairwise comparisons test with Benjamini–Hochberg adjustment (Benjamini and Hochberg 1995) was performed with 'FSA' package in R (Ogle et al. 2022) to determine which groups are statistically different. A Wilcoxon rank sum test was used to compare day 14 mortality between aged and fresh baits. All mortality analyses were performed using R (version 4.2.2) (R Core Team 2022) within RStudio (version 2022.7.1.554) (RStudio Team 2022).

Results

The initial weight of 5 hydrogel beads was highly uniform (0.31 ± 0.001 g, mean ± SEM, n = 90). An ANOVA revealed a significant difference in final weight among treatments (F = 672.04, df = 5, P < 0.0001). Post hoc analysis indicated the following. First, potassium sorbate (PS) reduced the hydrogel swelling (e.g., C vs. C + PS) (Table 1). Secondly, boric acid (BA) also reduced the swelling of the hydrogel beads [e.g., BA(O) and BA(S) vs. C]. Third, when PS and BA were both present, final weight of the hydrogel beads was smallest [e.g., BA(O) + PS and BA(S) + PS]. Interestingly, the final weight of hydrogel beads was consistently greater in the treatments with BA(O) than those with BA(S) (Table 1).

For the efficacy test, ant mortalities became noticeable on day 2 and gradually increased over time. By day 14, the total numbers of dead ants were 430 ± 37, 410 ± 38, 394 ± 29, 371 ± 40, 9 ± 1, and 17 ± 3 (mean ± SEM), for BA(O), BA(O) + PS, BA(S), BA(S) + PS, C, and C + PS, respectively. A Kruskal–Wallis test revealed a significant difference among treatments (H = 52, df = 5, P < 0.0001) (Fig. 2). A Dunn's all-pairwise comparisons test indicated that all boric acid treatments were similar in their efficacy, while being significantly different from controls (Fig. 2). Thus, BA(O) + PS was chosen for the subsequent test with aging.

When the aged bait [BA(O) + PS] was tested, the total number of dead ants by day 14 was 167 ± 12 (mean ± SEM, n = 12). When compared with the mortality data from fresh BA(O) + PS, the distributions in the two groups differed significantly (Wilcoxon rank sum test; W = 5, P = 0.0002 two-tailed), indicating that 2-month aged hydrogel bait was less effective than the fresh bait (Fig. 3).

Discussion

Hydrogel bait conditioned with 1.0% boric acid and 25% sucrose water caused significant mortality (77–84%) of Argentine ants by day 14. Technical Powder Optibor [BA(O)] and Boric Acid ReagentPlus [BA(S)] were similarly effective against Argentine ants when incorporated in the hydrogel. The addition of potassium sorbate preservative did not influence the insecticidal activity of boric acid delivered in the hydrogel. Qin et al. (2017) also reported that potassium sorbate [0.2–1% (wt/vol)] was effective in extending the shelf life of liquid baits without being repellent for ants. However, potassium sorbate affected the degree of swelling and thus, potentially, the economy of hydrogel bait. For example, the addition of preservatives may reduce the size of the hydrogel beads and thereby increase the amount of raw materials (e.g., sodium alginate) required to treat large areas. Future research is warranted to explore other preservatives (e.g., sodium benzoate, parabens) for their impacts on bait preservation and swelling. Interestingly, two different boric acid products were slightly different in terms of their impact on hydrogel swelling. This could be caused by some differences in impurities (e.g., 99.9% vs. 99.5% purity). The swelling capacity of hydrogels is known to be affected by type and concentration of salts in the swelling medium (Mirdarikvande et al. 2014, Wang et al. 2018). The differences in swelling warrant additional research.

Laboratory experiments with 2-month-old hydrogel bait [BA(O) + PS] indicated that the aged bait was significantly less effective than the fresh bait even with potassium sorbate preservative. With the storage conditions used in the current study, the aged hydrogel bait was about half as effective as the fresh hydrogel bait. The aged bait might be less palatable than fresh bait, causing the ants to consume less boric acid. Also, the long-term storage might have directly affected the availability/effectiveness of boric acid. For example, boric acid might have formed complexes with sucrose, potassium sorbate, or calcium alginate, putting the boron in less toxic or less available forms. Klotz et al. (2002) reported that adding certain sugar alcohols, such as sorbitol, to the bait could reduce the efficacy of boric acid (Klotz et al. 2002). Boric acid is known to form esters with the hydroxyl groups of sugars (Woods 1994), and alginate polymer possesses numerous free hydroxyl groups distributed along the backbone (National Center for Biotechnology Information 2022). Under certain conditions, boric acid readily forms complexes with the diols of alginate (Demey-Cedeño et al. 2014). Duration of storage might affect the extent of these possible chemical interactions and additional research is warranted.

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Author Contributions

BL: Conceptualization-Equal, Data curation-Equal, Formal analysis-Equal, Investigation-Equal, Methodology-Equal, Validation-Equal, Visualization-Equal, Writing – original draft-Equal, Writing – review & editing-Equal. **HP:** Data curation-Equal, Methodology-Equal, Writing – original draft-Equal. **KC:** Data curation-Equal, Methodology-Equal, Writing – original draft-Equal. **MR:** Investigation-Equal, Supervision-Equal, Writing – original draft-Equal, Writing – review & editing-Equal. **C-YL:** Investigation-Equal, Supervision-Equal, Writing – original draft-Equal, Writing – review & editing-Equal. **D-HC:** Conceptualization-Equal, Data curation-Equal, Formal analysis-Equal, Funding acquisition-Equal, Investigation-Equal, Methodology-Equal, Project administration-Equal, Resources-Equal, Software-Equal, Supervision-Equal, Validation-Equal, Visualization-Equal, Writing – original draft-Equal, Writing – review & editing-Equal.

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